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BIODETECTION GRINDER

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16. ABSTRACT

One method of sampling materials for detection of embedded organisms utilizes the crushing action of a Mill Blender. Destruction of microorganisms is high and decreases accuracy of the biotests.

To improve upon this method and device, a Biodetection Grinder was developed. It is a device that employs a shearing action to generate controllable, sized particles with a minimum of energy input.

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TABLE OF CONTENTS

	Page
INTRODUCTION	1
DESCRIPTION OF OPERATIONS	2
MECHANICAL EVALUATION OF PROTOTYPE BIODETECTION GRINDER	5
BIOLOGICAL EVALUATION OF SAMPLES PRODUCED FROM THE BIODETECTION GRINDER	6
Pellet Formation	7
Collection and Treatment of Pellets and Grindings	9
Culture Techniques	10
Particle Sizing of Rigidax and Lucite	10
Results	11
Discussion	15
Conclusion	16
APPENDIX A — OPERATION PROCEDURE FOR BIODETECTION GRINDER	18
BIBLIOGRAPHY	24

LIST OF ILLUSTRATIONS

Figure	Title	Page
1.	Biodetection Grinder (Prototype)	3
2.	Biodetection Grinder (Second Generation)	4
3.	Percent distribution of Rigidax grindings in micron size range with No. 100 wheel	12
4.	Percent distribution of Lucite grindings in micron size range with No. 100 wheel	14

LIST OF TABLES

Table	Title	Page
1.	Particle Size Distribution in Percentage	7
2.	Assay of Rigidax Pellets to Show Uniform Distribution	11
3.	Percent Recovery of Organisms From Rigidax Capsules After Grinding	12
4.	Assay of Lucite Pellets to Show Uniform Distribution	13
5.	Percent Recovery of Organisms From Lucite Capsule After Grinding	14
6.	Assay of Spore Strips to be Used as a Baseline for Determining the Percent Recovery From Castoglas Pellets	15
7.	Pellets Percent Recovery of Organism From Castoglas After Grinding	16

TECHNICAL MEMORANDUM X-64765

BIODETECTION GRINDER

INTRODUCTION

To determine the presence of microbial life in outer space and to determine whether spacecraft components sent into outer space are sterile, a method of checking for embedded as well as surface microorganisms is necessary. Embedded microorganisms survive in some hard materials to great depths and sampling has to be made at the proper depth or cross section for accurate evaluations. Also, some aerospace materials have to be reduced to a size within 1 to 8 microns in order to obtain the best biological assay.

Conventional grinding cannot be used to obtain these sizings because the high energy level results in heating the particles high enough to kill living organisms. Also, the shearing action contributes to the reduction of microbial life. It is not feasible, or may not be possible, to accurately measure the maximum temperature of the micro particles because of size and cooling rate. Therefore, empirical methods are used to establish the best sampling techniques.

One of the present methods of sampling aerospace materials for detection of embedded organisms utilizes the crushing action of a Pica Mill Blender. Destruction of microorganisms using this method is high and decreases the accuracy of the tests.

With this background data, a program was initiated for the development of a sampling device to reduce the particles to the required size without killing the microorganisms. The result was the development of the Biodetection Grinder, a device that employs a shearing action with a minimum energy input to generate the particles. Using this device the desired particle sizes were obtained in materials ranging from soft plastics to hard rocks.

This report describes the development of the prototype Biodetection Grinder, its operation, and results of laboratory evaluation of selected aerospace components. A second generation version of the grinder was constructed and is presently in use at the Communicable Disease Center in Phoenix, Arizona. A production version, which modified the second generation type to provide faster particle reduction rates, was developed and transferred to the Communicable Disease Center for further evaluation.

DESCRIPTION OF OPERATIONS

These operating characterisitcs apply to the prototype, second generation, and production grinder.

Cutting or grinding of the specimen material is done under a slight pressure in a sealed transparent chamber. The specimen is held by a chuck type clamp mounted on a slide which moves horizontally back and forth. Coupling of the drive shaft to the slide is such that rotary motion of the drive shaft results in reciprocating motion to the slide and chuck. A 100 rpm, 27 Vdc motor provides the driving power for the reciprocating chuck as well as the grinding wheel and the linear feed subassembly.

The grinding wheel drive shaft, motor and coupling arrangement provide a variable controlled linear feed of the grinder into the specimen material. The grinder is attached to the drive motor with an adapter which permits quick disconnects for replacement purposes so that different grit size grinding wheels may be used.

Linear movement of the grinder and its drive motor subassembly is accomplished through a gear reduction mechanism which is driven by a variable speed motor. The gear reduction mechanism controls the forward movement of the cutter to 0.0001 in. per revolution, and the total linear movement is from 0 to 1 in.

Fines from the sample may be collected in a polyethylene bag for aseptic storage as shown in Figure 1. The polyethylene bag may be replaced by a petri dish if immediate bioassay of the fines is desirable.

Improvements in the second generation grinder shown in Figure 2 were as follows:

1. A limit switch arrangement was incorporated to provide automatic cutoff of linear motion at the desired increment settings.

2. A linear travel indicator showed the amount of material removed from specimens, thereby eliminating the need for weighing fines.

3. An automatic sealing mechanism was incorporated to aseptically seal the polyethylene bag.

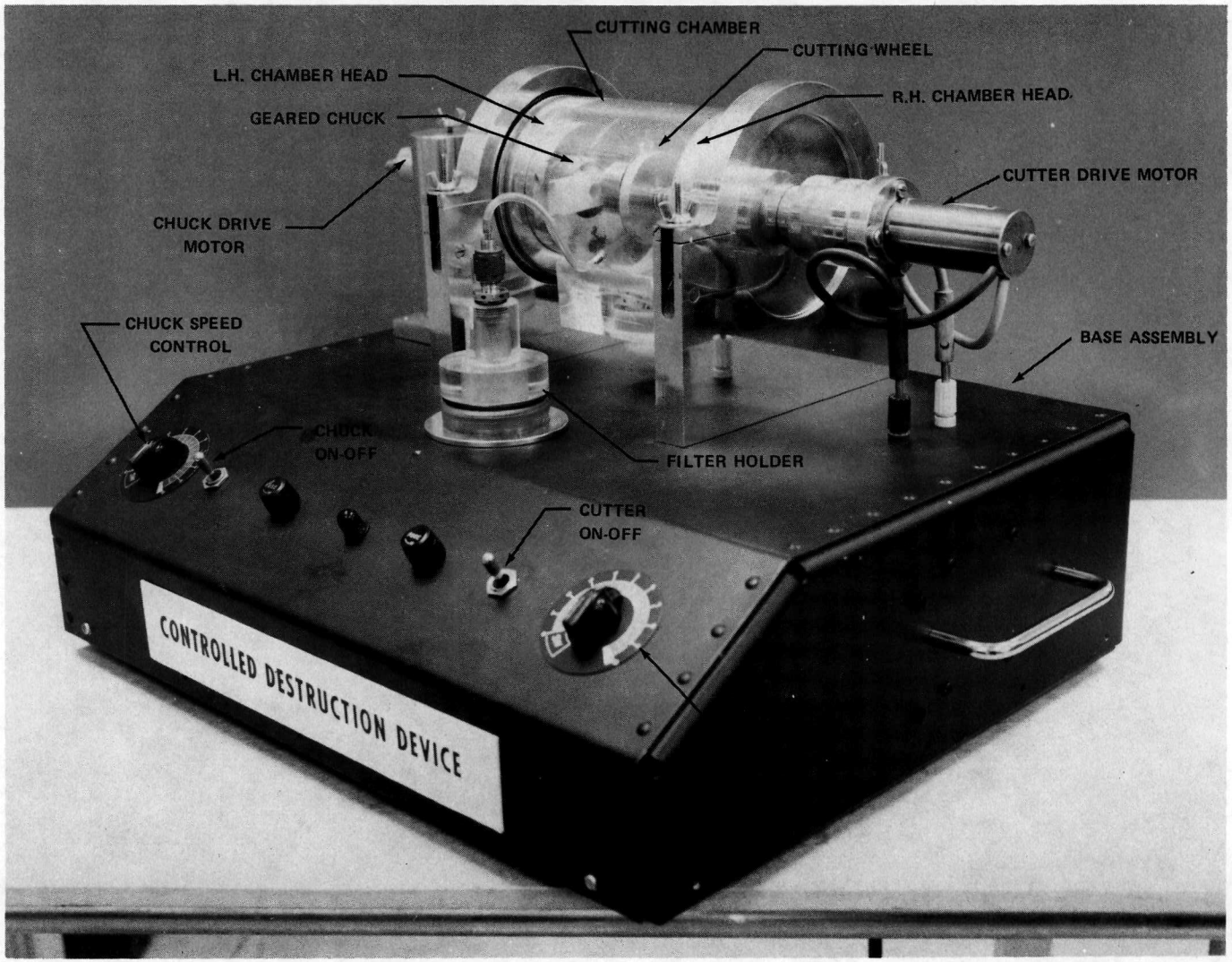

Figure 1. Biodetection Grinder (Prototype).

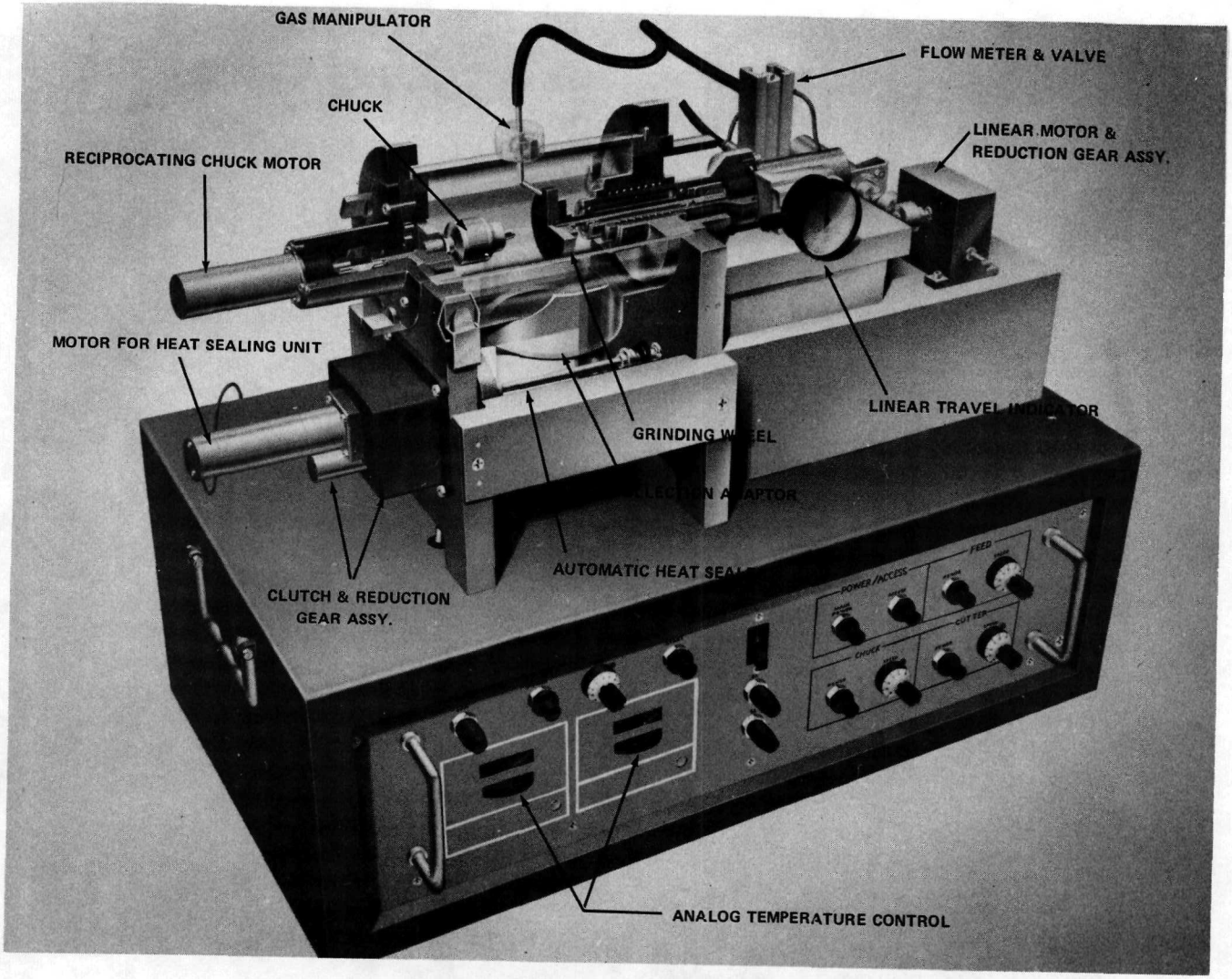

Figure 2. Biodetection Grinder (Second Generation).

4. A port was provided in the top of the cutting chamber directly above the specimen to introduce sterile gas, air, or cooling liquid directly on the specimen. An air stream from the port could be used to direct the flow of specimen fines toward the collector.

Specific changes incorporated in the production version were:

1. The aseptic chamber was not needed and was replaced by a Plexiglas hood because the grinder was used in a laminar flow clean room.

2. The fines from the grinding wheel are collected directly in a sterile beaker, eliminating the need for the particle collector and bag sealer. By removing the bag sealer, it was possible to place an adjustable platform under the grinding wheel to support the beaker.

3. To reduce the time required to sterilize the cutting wheel and single arbor, several slip-in arbors and snap-on cutting wheels are used, permitting sterilization of several units at one time while several other units are in operation.

4. A micrometer-set, automatic shut-off device was included to permit presetting the grinder to take a certain size sample, at the completion of which the machine is automatically stopped.

MECHANICAL EVALUATION OF PROTOTYPE BIODETECTION GRINDER

A predictable size range of particles from a wide variety of materials is required for accurate analysis of embedded microorganisms. Tests were conducted to determine how well the Biodetection Grinder met this requirement. Nine specimens of varying hardness were ground and sized. The specimens were identified as follows:

Specimen No.	Material
1	Resistor, Brass Core
2	Resistor, Copper Core

Specimen No.	Material
3	Resistor, Micarta
4	Resistor, Steel Core
5	Aluminum Alloy 6061-T6
6	Brass, Hard Navy
7	Lucite
8	Rigidax
9	Resistors, Graphite Core

Attempts were made to grind two additional materials without satisfactory results. Grinding of Epoxy C-7 resulted in loading the cutting wheel with the specimen material, thereby reducing the ability of the grinder to control particle size. Careful selection of grinding parameters reduces the wheel loading problem, but further research is needed to provide satisfactory grindings for materials of this nature.

Silicone rubber (RTV 11) is an extremely resiliant material. During grinding, this material tends to break up into relatively large clumps. Restraining this specimen in a rigid tube results in a controlled grinding process; however, it should be noted that the use of a tube restraint introduces a contaminant into the specimen material. Therefore, care should be exercised in selecting a restraint material that is nonbacteriostatic.

Table 1 shows the distribution of particle sizes for the nine materials ground. All tests were made with a linear feed pressure of 2.5 pounds and a cutting wheel of 100 grit.

BIOLOGICAL EVALUATION OF SAMPLES PRODUCED FROM THE BIODETECTION GRINDER

To establish baseline data for the Biodetection Grinder, biological model systems were made from Rigidax, a soft, water-soluble plastic; Lucite, a hard, acetone-soluble plastic; and Castoglas, a hard, clear, insoluble plastic.

TABLE 1. PARTICLE SIZE DISTRIBUTION IN PERCENTAGE

Specimen Number	Particle Size Range (microns)					Cutter Speed (rpm)	Chuck Speed (stokes/min)
	>100	50-100	25-50	5-25	3-5		
1	0.13	0.50	1.92	38.12	59.33	12	10
2	0.5	0.25	1.37	41.59	56.29	15	10
3	0.40	0.84	3.47	38.51	56.76	25	20
4	0	0.21	0.99	20.38	77.69	15	10
5	4.25	0.53	3.72	21.27	70.21	12	10
6	7.17	2.05	2.73	23.84	64.16	25	10
7	0.57	0.23	0.86	39.23	59.11	30	20
8	5.26	6.58	3.29	47.37	37.50	30	15
9	0	0.45	1.37	37.50	60.68	12	10

Pellets made of Rigidax and Lucite were impregnated with spores of Bacillus subtilis var niger, assayed for uniform spore distribution, and ground in the Biodetection Grinder. The grindings were cultured and percent recovery calculated. Grindings were also measured and size percentage ranges were established for the number 100 grit grinding wheel.

The Castoglas pellets embedded with Bacillus subtilis and Bacillus stearothermophilus impregnated spore strips were ground on the Biodetection Grinder. The grindings were cultured and percent recovery calculated. Several spore strips were cultured individually to determine the actual number of spores impregnated on each strip. This determination was used as a baseline to calculate the percent of microorganisms recovered from the grindings.

Pellet Formation

Rigidax. This is a semihard, gray, water-soluble plastic which melts at 70°C and sets at about 40°C. Pellets contaminated with spores of Bacillus subtilis were prepared in a class 100 clean bench by weighing 10 grams of

Rigidax in an aluminum pan. The lyophilized spore suspension was suspended in 1 ml 95 percent ethanol and ultrasonicated to break up clumps. This was verified by spore stains.

Rigidax was melted and the spore suspension was added. A sterile wooden stick was used to stir the mixture which was allowed to bubble gently until evaporation of the ethanol appeared complete. Overheating was avoided so that maximum spore survival was attained. Liquid Rigidax was immediately poured into No. 00 gelatin capsules and placed in a refrigerator at 4°C to harden thoroughly.

After 2 hours, the capsules were removed from the refrigerator, scored with a sterile, heated scalpel and the gelatin covering discarded. The Rigidax pellets were handled with a gloved hand and transferred to the freezer for storage at -15°C in sterile plastic bags.

Lucite. This is a hard, clear acetone soluble plastic which is made from two components and set at 50°C over a period of 6 hours.

Pellets approximately 0.63 cm (0.25 in.) in diameter were prepared in a class 100 clean bench as follows:

1. Removal of Inhibitor. Methyl Methacrylate Liquid (MML) contains a polymerization inhibitor which was removed in the following manner:

One portion of MML was washed twice in the separatory funnel with two successive portions of 2 percent NaOH. The first washing gave a dirty pink color; the second wash was clear.

The MML was then washed with two successive portions of distilled water. The MML formed the top layer in each case.

The MML was then transferred to a glass stoppered bottle and 10 gm Na_2SO_4 were added per 100 ml of washed MML to remove any remaining water. This mixture was stored at 4°C until needed.

2. Contamination of Methyl Metacrylate Powder (MMP). Ten gm of MMP were weighed in an aluminum pan. A lyophilized suspension containing 6×10^6 Bacillus subtilis spores was suspended in 95 percent ethanol and ultrasonicated 12 min to break up clumps of spores. Absence of clumping was verified by spore stains. Operating in the clean bench, the spore suspension was added to the 10 gm of MMP, mixed with a sterile wooden stick, and allowed to dry for 16 hours in the clean bench.

3. Preparation of Mixture of MMP and MML. When the contaminated MMP was thoroughly dry, 8 ml of the MML were added and stirred gently with a sterile wooden stick. This mixture was placed in a vacuum jar and 28 in. Hg vacuum was pulled slowly, causing bubbles of gas to evolve from the plastic. When bubbles ceased to evolve, the mixture was removed and gently stirred to make a clear casting. The mixture was poured in No. 00 capsules and placed in a 50°C oven for 6 hours.

The pellets were placed in sterile water to allow the capsules to dissolve. The pellets were removed and stored in sterile plastic bags at -15°C.

Castoglas. Clear, hard, insoluble plastic and Castoglas pellets were contaminated with spore strips. One spore strip was rolled into a circle and placed in the bottom of each gelatin capsule. Castoglas, made according to directions on the can, was poured into each capsule and allowed to harden for 2.5 hours at room temperature. The gelatin capsules were then removed with a heated scalpel. Each pellet was put into a separate plastic bag and stored in the refrigerator until needed.

Collection and Treatment of Pellets and Grindings

Pellets

Metrology. Each pellet was weighed on a balance with a 0.001 gm sensitivity and measured with a micrometer.

Storage. Each pellet was identified in separate bags by a number, weight, and length and was stored at -15°C until needed.

Pellet Remaining After Grinding

Metrology. The Lucite and Rigidax pellets remaining in the chuck after grinding were weighed, measured, and stored in an identified plastic bag at -15°C until ready for culture. It was not necessary to weigh the remaining Castoglas pellets since the material was insoluble and could not be cultured.

Grindings

Metrology. Grindings were collected in preweighed plastic bags so that the weight of the grindings could be determined after collection without removal from the collection bags. The grindings were stored at -15°C until cultured.

Culture Techniques

Rigidax. To determine uniform spore distribution throughout the Rigidax pellets, two pellets were cut into two pieces. Each piece was weighed, then dissolved in sterile distilled water to give a 1:100 dilution. Serial 1:100 dilutions were made and plated in duplicate by standard pour plate methods.

Seven Rigidax pellets were ground by the Biodetection Grinder. The grindings and the pellets remaining after grinding were dissolved in sterile distilled water according to weight, to give 1:100 dilution. Serial 1:10 dilutions were made and plated in duplicate by standard pour plate methods.

Colony counts were determined on all pour plates after 24 hours incubation at 35°C.

Lucite. To determine uniform spore distribution throughout the Lucite pellets, four pellets were cut into two pieces. Each piece was weighed then dissolved by weight in sterile acetone to give a 1:100 dilution. Serial 1:10 dilutions were made and plated in duplicate by standard pour plate methods.

Colony counts were determined on all pour plates after 48 hours incubation at 35°C.

Castoglas. To determine the number of spores impregnated on the spore strips embedded in the Castoglas pellets, three spore strips were placed individually into 10 ml of trypticase soy broth, ultrasonicated for 12 min and incubated for 3 hours at 35°C. After incubation, serial dilutions of 10^3, 10^4, and 10^5 were made and 1 ml of each dilution was plated in duplicate with trypticase soy agar. The number of colonies was counted after 24 hours of incubation at 35°C. These colony counts were used as a baseline for determining the percent recovery from the grindings.

The end of the pellet containing the rolled spore strip was completely ground. One-tenth gm of the grinding was placed into 10 ml of trypticase soy broth and cultured (in the same manner as the spore strips in the preceding paragraph). The colony count data obtained from the spore strips and the grindings were used to compute the percent recovery.

Particle Sizing of Rigidax and Lucite

Slide Preparation. After grindings had been removed for culturing, a small amount of grindings was suspended in 5 ml of Freon and ultrasonicated for 1 min to disperse clumps. A slide was prepared from grindings representative of each pellet by placing one drop of the ultrasonicated material on

alcohol flamed slide and allowing the Freon to evaporate. This was covered with a flamed coverslip and microscopically examined at 500X.

Microscopic Sizing. Particles were sized and percentages calculated by measurement with a calibrated ocular micrometer at 500X. Particles were sized from 2 to 4 microns, 4 to 8 microns, and greater than 8 microns. The number of particles counted on the thinnest section of each slide was 100.

Results

Rigidax. It was found that spores had been uniformly distributed throughout the pellets assayed for this purpose (Table 2). Recovery of spores from grindings compared with recovery from the remaining pellet is shown in Table 3. Size range of the grindings is shown in Figure 3.

TABLE 2. ASSAY OF RIGIDAX PELLETS TO SHOW UNIFORM DISTRIBUTION

Sample[a]	Replicate	Standard Pour Plate Count	Average Pour Plate Count
1a	1	5.52×10^6	5.56×10^6
	2	5.60×10^6	
1b	1	5.40×10^6	5.37×10^6
	2	5.34×10^6	
2a	1	5.78×10^6	5.55×10^6
	2	5.33×10^6	
2b	1	5.57×10^6	5.51×10^6
		5.46×10^6	

a. a and b refer to sections of the same pellet.

TABLE 3. PERCENT RECOVERY OF ORGANISMS FROM RIGIDAX CAPSULES AFTER GRINDING

Sample	Pellet After Grinding, Standard Pour Plate Count	Grindings, Standard Pour Plate Count	Percent Recovery of Organisms
1	7.00×10^6 7.40×10^6	6.70×10^6 6.69×10^6	93
2	6.90×10^6 6.69×10^6	6.54×10^6 6.25×10^6	94
3	8.26×10^6 7.83×10^6	8.03×10^6 7.98×10^6	99
4	6.18×10^6 5.60×10^6	5.38×10^6 5.16×10^6	89
5	7.31×10^6 6.97×10^6	7.26×10^6 7.10×10^6	100
6	5.56×10^6 5.49×10^6	5.32×10^6 5.30×10^6	96
7	6.13×10^6 6.27×10^6	5.80×10^6 5.46×10^6	92

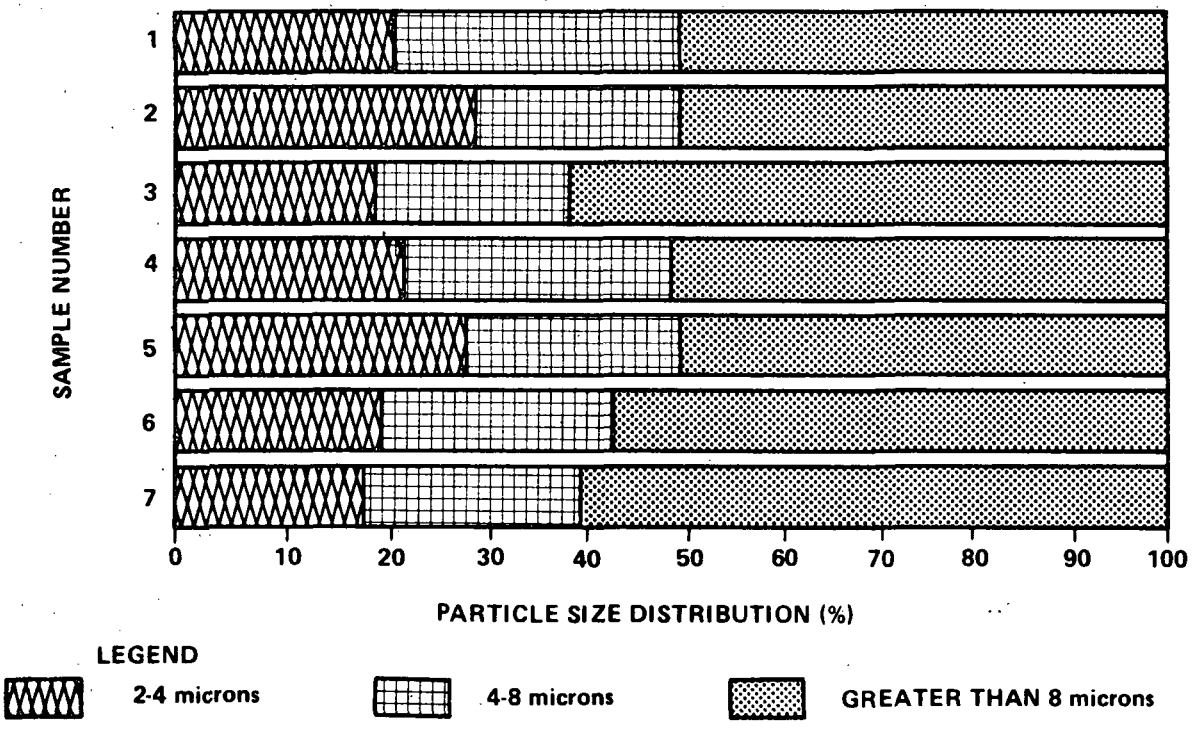

Figure 3. Percent distribution of Rigidax grindings in micron size range with No. 100 wheel.

Lucite. Cultures of pellets cut in pieces indicate that spore distribution in Lucite varied throughout the pellet as shown in Table 4. Spore recovery from grindings compared with recovery from the remaining pellet is shown in Table 5. Size range of the grindings is shown in Figure 4.

Castoglas. Since the entire spore strip in the end of each pellet was ground, the pellet was considered to have a uniform distribution of organisms. Table 6 is included to indicate the average pour plate count for three spore strips that were not embedded in Castoglas. Percent recovery of the 10 samples tested varied with each pellet as shown in Table 7.

TABLE 4. ASSAY OF LUCITE PELLETS TO SHOW UNIFORM DISTRIBUTION

Sample[a]	Replicate	Standard Pour Plate Count	Average Pour Plate Count
1a	1	1.17×10^6	1.20×10^6
	2	1.23×10^6	
1b	1	1.00×10^6	1.03×10^6
	2	1.05×10^6	
2a	1	1.70×10^5	1.40×10^5
	2	1.10×10^5	
2b	1	3.3×10^5	3.7×10^5
	2	4.1×10^5	
3a	1	8.3×10^5	9.0×10^5
	2	9.7×10^5	
3b	1	10.0×10^5	9.9×10^5
	2	9.8×10^5	
4a	1	7.2×10^5	7.1×10^5
	2	7.0×10^5	
4b	1	3.6×10^5	3.4×10^5
	2	3.1×10^5	

a. a and b refer to sections of the same pellet.

TABLE 5. PERCENT RECOVERY OF ORGANISMS FROM LUCITE CAPSULE AFTER GRINDING

Sample	Pellet After Grinding, Standard Pour Plate Count	Grindings, Standard Pour Plate Count	Percent Recovery of Organisms
1	8.0×10^5 7.4×10^5	1.9×10^5 2.1×10^5	26
2	6.4×10^5 7.2×10^5	2.1×10^5 2.6×10^5	33
3	6.8×10^5 7.5×10^5	6.0×10^4 6.0×10^4	8
4	5.8×10^5 6.0×10^5	2.6×10^5 3.0×10^5	47
5	2.0×10^5 1.9×10^5	1.0×10^5 1.2×10^5	50
6	4.7×10^5 4.2×10^5	3.0×10^4 3.0×10^4	7
7	5.5×10^5 4.8×10^5	8.0×10^4 9.0×10^4	17
8	4.0×10^5 3.9×10^5	1.9×10^5 2.3×10^5	53

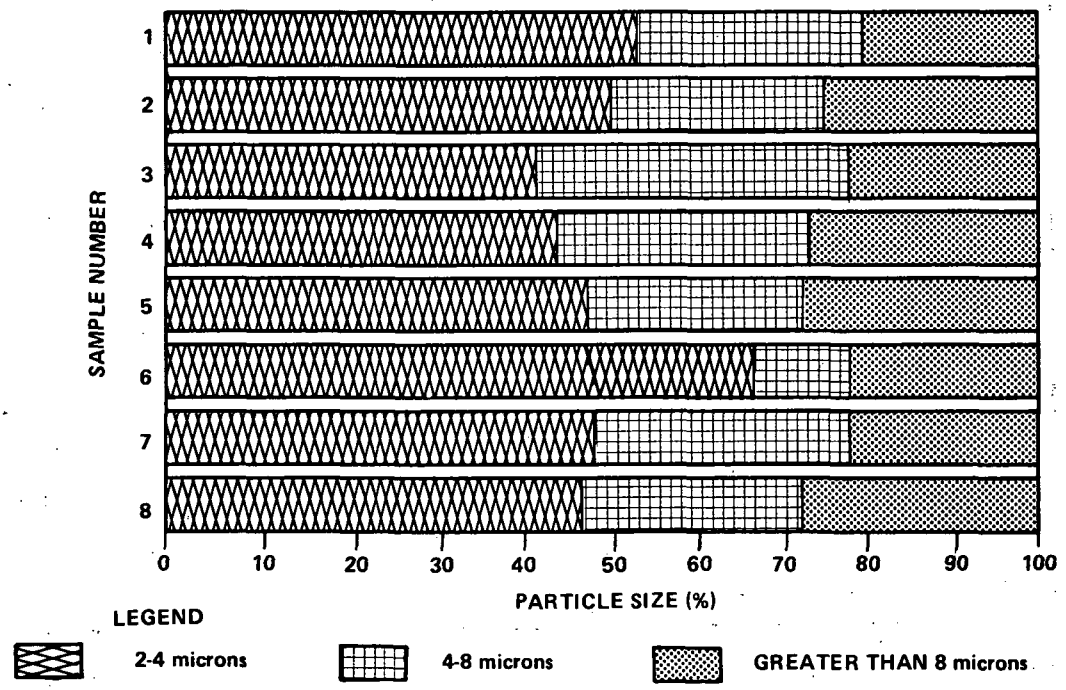

Figure 4. Percent distribution of Lucite grindings in micron size range with No. 100 wheel.

TABLE 6. ASSAY OF SPORE STRIPS TO BE USED AS A BASELINE FOR DETERMINING THE PERCENT RECOVERY FROM CASTOGLAS PELLETS

Sample	Replicate	Standard Pour Plate Count	Average Pour Plate Count
1	1	2.3×10^5	2.4×10^5
	2	2.5×10^5	
2	1	2.15×10^5	2.08×10^5
	2	2.02×10^5	
3	1	2.43×10^5	2.35×10^5
	2	2.27×10^5	

Discussion

Rigidax. Rigidax is an easily impregnated material which, when ground, produces a high percentage of greater-than-8-micron-size particles. This was expected because Rigidax has a tendency to flake even when ground slowly. Reproducibility of particle size was good. Spore distribution was uniform throughout the pellet. This was reflected by the consistency of recovery of high percentages of organisms from the grindings.

Lucite. Lucite was more difficult than Rigidax to uniformly impregnate with spores. This was seen by the results of assaying pellets cut in half. The erratic results obtained from cultures of the grindings was most likely due to nonuniform impregnation of the Lucite pellets. Lucite produced a high percentage of particles in the 2 to 4 micron range.

Castoglas. Spores were uniformly distributed in the Castoglas pellets by the incorporation of commercial spore strips in the ends of the pellets. The variability of the percent recovery of the spores from the Castoglas was possibly due to the insolubility of Castoglas. Some spores remained trapped in the plastic where they could not come in contact with the nutrient media; thus, the spores could not germinate.

TABLE 7. PELLETS PERCENT RECOVERY OF ORGANISM FROM CASTOGLAS AFTER GRINDING

Sample	Replicate	Standard Pour Plate Count × 10^5	Average Pour Plate Count × 10^5	Recovery
1	1	1.10	1.08	47.4
	2	1.06		
2	1	0.91	0.905	39.6
	2	0.90		
3	1	1.36	1.29	56.6
	2	1.23		
4	1	0.97	0.935	41.0
	2	0.90		
5	1	0.68	0.69	30.3
	2	0.70		
6	1	0.71	0.725	31.8
	2	0.74		
7	1	0.43	0.455	20.0
	2	0.48		
8	1	1.45	1.43	62.7
	2	1.41		
9	1	0.88	0.855	37.5
	2	0.83		
10	1	0.94	0.965	42.3
	2	0.99		

CONCLUSION

Test results using the Biodetection Grinder and three biological model systems show that the Biodetection Grinder can produce grindings in a predictable size range. Further, these results indicate that reproducible recovery rates are dependent on the type of material used for making the biological test models.

Additional tests were performed to produce uniformly contaminated biological models using different materials to demonstrate the efficiency of the Biodetection Grinder. While work was carried out to produce these models, actual spacecraft components were ground and assayed for viable organism content.

APPENDIX A

OPERATION PROCEDURE FOR BIODETECTION GRINDER

SCOPE

This set of procedures is designed to instruct assay laboratories in the aseptic operation of the Biodetection Grinder using materials contaminated with spores of Bacillus subtilis. These instructions are based on operating procedures currently in use by this laboratory for the Prototype Grinder.

EQUIPMENT

Tools needed for the operation, adjustment, and cleaning of the grinder are as follows:

Jacobs chuck key, 0.63 cm (0.25 in.).

6 in. screwdriver with a 0.63 cm (0.25 in.) blade.

Wire brush.

1.3 cm (0.5 in.) round camel hair brush.

Equipment utilized for maintenance of aspetic techniques and contamination control are:

Class 100 clean bench.

Ethylene oxide sterilizer.

Sterile forceps.

Sterile 4 mil polyethylene bags, 9.5 cm (3.75 in.) wide by 7.6 cm (3 in.) long.

Sterile gloves.

Sterile screwdriver.

Sterile 0.63 cm (0.25 in.) Jacobs chuck key.

Formaldehyde, 5 percent in 70 percent isopropyl alcohol.

Sterile towel, 45.7 cm by 45.7 cm (18 by 18 in.).

Rodac plates filled with trypticase soy agar.

Isopropyl alcohol, 70 percent.

Unispore spore strips.

Biodetection Grinder.

GRINDER ASSEMBLY

The following procedure should be used to assemble the Grinder:

- Fit chuck-motor assembly in one end of grinding chamber.
- Attach appropriate grit grinding wheel to shaft with three screws (provided).
- Insert shaft into grinder-motor housing and push with a twisting motion until a click is heard.
- Fit grinder-motor housing assembly into the end of the grinding chamber opposite the chuck.
- Place grinding collection receptacle in the hole in the bottom of the grinding chamber.
- Insert spore strip impregnated with Bacillus subtilis spores in the opening for the collection bag.
- Place the entire assembly in a 4 mil pastic bag 61 cm by 20 cm (24 in. by 8 in.). Insert a cotton wick in the end, secure with a rubber band and a piece of ethylene-oxide-sensitive tape.

STERILIZATION

Equipment is sterilized by the following procedures:

- The assembled grinding chamber is sterilized using 12 percent ethylene oxide and 88 percent Freon in an air displacement cycle at 54.4°C (130°F) for 16 hours.
- After completion of the sterilization cycle, the unit (in its sterilization bag) is placed in a class 100 clean bench with the blower operating and allowed to degas for 24 hours.
- Polyethylene bags 8.9 by 7.6 cm (3.5 by 3 in.), 4 mil thick are packaged singly in paper pouches, sterilized with the grinding chamber, and degased. A spore strip is inserted in the paper pouch prior to sealing.
- All other sterile tools are packaged in one paper pouch and sterilized as specified above.

ASEPTIC INSERTION OF BIOMODEL PELLET

The specimen to be ground is inserted in the Grinder by the following method:

- Wipe the inside of the class 100 clean bench with 70 percent isopropyl alcohol to decontaminate.
- Place four Rodac plates in the clean bench.
- Place the sterile towel on the work surface of the clean bench.
- Open the bag containing the grinding chamber in the clean bench and place the chamber on the sterile towel.
- Open the bags containing sterilized tools and collection bag and place the contents on the sterile field.
- Aseptically remove the specimen to be ground from its storage bag and place it on the sterile field.

- Donn sterile gloves.
- Remove and culture all spore strips.
- Using the sterile screw driver, aseptically remove the chuck-motor assembly.
- Place specimen in chuck and tighten chuck with sterile Jacobs chuck key.
- Replace chuck-motor assembly in end of grinding chamber.
- Place specimen bag on specimen collector.
- Remove sterile gloves and sterile assembly material.

The grinder chamber is now handled as a closed sterile item.

THE GRINDING OPERATION

The steps listed should be used to grind a specimen.

- Check to see that all power and drive switches are off.
- Plug power cords into 115 V receptacles and grinder power source.
- Turn on all main power switches.
- Adjust speed and feed controls to the desired rates.
- Grind specimen.
- Shut off all switches, disconnect power cords.
- Remove grinder chamber top mounts.
- Loosen grinder connector screw.
- Remove grinder chamber from power source.
- Place grinder chamber on towel.

- Remove collection bag using aseptic technique, fold top edge in and seal with cellophane tape.

- Remove chuck-motor housing and loosen chuck.

- Using sterile forceps, transfer remainder of specimen back to its storage container.

PREPARATIONS FOR REASSEMBLY OF GRINDING CHAMBER

The following procedure should be used to reassemble the Grinder.

- Using screwdriver, remove grinder-motor housing.

- Using the 1.3 cm (0.5 in.) camel hair brush, remove any particles of the specimen adhering to parts of the grinder or chamber. Be certain that all excess grindings cleaned from the mechanism are collected on the towel.

- After cleaning the housing, use the wire brush to clean the grinding wheel. Reassemble grinder.

- Remove towel from the work area and autoclave.

- Wipe all surfaces in the clean bench with the formaldehyde/alcohol mixture.

QUALITY ASSURANCE

To assure good results, one should confirm compliance with the following practices:

- Spore strips impregnated with Bacillus subtilis spores and packaged in glassine envelopes (Unispore, Castle Co.) are included in each separate package of articles to be sterilized by ethylene oxide.

- Spore strips are removed from the sterilized packages and cultured according to manufacturer's instructions.

- Rodac plates filled with trypticase soy agar are placed in the clean bench to aid in detection of external contamination.

- If the sterile assembly-grinding procedure requires longer than 1 hour, Rodac plates are replaced at the rate of 4 per hour to prevent excessive drying of the culture medium.

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APPROVAL

BIODETECTION GRINDER

By F. J. Beyerle

The information in this report has been reviewed for security classification. Review of any information concerning Department of Defense or Atomic Energy Commission programs has been made by the MSFC Security Classification Officer. This report, in its entirety, has been determined to be unclassified.

This document has also been reviewed and approved for technical accuracy.

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8/7/73

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